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THE RELEASE OF HISTAMINE BY PARASYMPATHETIC STIMULATION IN GUINEA-PIG AURICLE AND RAT ILEUM

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SUMMARY

1. Two preparations, a segment of rat ileum and the vagally innerved guinea-pig auricles, have been used in an analysis of the responses to vagal or to electrical field stimulation.

2. The responses to parasympathetic stimulation were depressed by atropine and by tetrodotoxin, and potentiated by eserine.

3. Supramaximal stimulation (10–20 Hz) resulted in increased release of acetylcholine and histamine, both in rat ileum and guinea-pig auricles.

4. The release of histamine after parasympathetic stimulation did not exhibit tachyphylaxis, and it was not reproduced by non-parasympathetic stimuli.

5. In both preparations, atropine produced a significant, dose-related reduction of histamine measured in the bath fluid after stimulation, while eserine increased histamine output.

6. A significant diminution of mast cell granules metachromasia was observed in guinea-pig auricles and in rat intestine after parasympathetic stimulation.

7. The possibility is discussed that acetylcholine released by parasympathetic stimulation would in turn evoke the secretion of histamine from tissue mast cells.

INTRODUCTION

Previous studies in our laboratories have shown that exogenous acetylcholine causes the release of histamine from isolated rat serosal mast cells, featuring a sequential exocytotic process, dependent on metabolic energy, and upon the extracellular concentration of sodium, calcium and hydrogen ions (Fantozzi, Masini, Blandina, Mannaioni & Bani-Sacchi, 1978*a*; Fantozzi, Moroni, Masini, Blandina & Mannaioni, 1978*b*; Blandina, Fantozzi, Mannaioni & Masini, 1980). The secretion of histamine is preferentially evoked by muscarinic cholinergic agonists, and competitively blocked by atropine, indicating the participation of muscarinic receptors. We

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have also reported the presence of specific [^{3}H]quinuclidinyl-benzilate binding sites in isolated, purified intact mast cells and in their crude membrane preparations (Fantozzi, Masini, Blandina & Mannaioni, 1979; Masini, Fantozzi, Blandina, Brunelleschi & Mannaioni, 1983), and a heterogeneous pattern of mast cell sensitivity to the cholinergic stimulus, ranging from a full reaction to nanomolar concentrations of acetylcholine to a virtual lack of response (Masini, Blandina, Fantozzi, Brunelleschi & Mannaioni, 1981).

However, these accounts of the histamine-releasing action of acetylcholine have not been reproduced in other laboratories as reported by Kiernan (1972), Kazimierczak & Diamant (1978), Foreman (1981).

The purpose of the experiments described in this paper was to examine the link between cholinergic nervous system and tissue histamine stores, in preparations physiologically more integrated than purified mast cells. With this aim, we have measured both the resting output of acetylcholine and histamine, and their release resulting from stimulation of the vagus nerve of isolated, vagally innerved guinea-pig auricles, or from the field stimulation of parasympathetic nerve endings of isolated ileal segments of the rat. We have selected these preparations from different animal species containing connective tissue mast cells and mucosal mast cells, in consideration of mast cell heterogeneity in response to the same stimulus (Bienenstock, Befus, Pearce, Denburg & Goodacre, 1982). A preliminary report of some of the results was communicated at the European Histamine Research Society Meetings (Blandina, Barattini, Fantozzi, Masini & Mannaioni, 1983; Blandina, Barattini, Fantozzi, Masini, Brunelleschi & Mannaioni, 1984).

METHODS

Isolation and perfusion of vagally innerved guinea-pig auricles

Guinea-pigs, of either sex, were stunned by a blow on the head and bled out. The thorax was opened, the aorta cannulated, and the heart was perfused *in situ* with oxygenated Tyrode solution at 37 °C by means of a Langendorff apparatus, after excision of all the cardiac vessels. This allowed the careful dissection of the right cervical vagus and the isolation of both the auricles with the right cervical vagus attached. The isolated, vagally innerved guinea-pig auricles were placed in a muscle chamber, and perfused with Tyrode solution of the following composition (mM): Na^+ 149·3; K^+, 2·7; Ca^{2+}, 1·8; Mg^{2+}, 1·05; Cl^-, 145·4; HCO_3^-, 11·9; $H_2PO_4^-$, 0·4; (+)glucose, 5·6 (Dieterich, Löffelholz & Pompetzki, 1977). The solution was kept at 37 °C and gassed with a mixture of 95 % O_2–5 % CO_2, giving a final pH of 7·48. The flow rate was adjusted by a peristaltic pump to 5 ml min^{-1}. The right cervical vagus nerve of the isolated auricle was stimulated (40 V, 1 ms) with platinum ring electrodes for 1 min at 20 Hz. The rate and contraction were recorded by means of a pressure transducer connected to an ink-writing oscillograph. Histamine and acetylcholine were measured in perfusates collected at 2 min intervals.

Preparation of rat intestinal muscle strips

Adult Wistar rats of both sexes were killed by exsanguination and the ileum excised. The terminal portion was used after the 10 cm nearest to the ileo-coecal junction had been discarded. Segments of rat ileum, 4 cm in length, were freed of mesentery and suspended in an organ bath containing 10 ml of a modified Krebs solution of the following composition (mM): Na^+, 143; K^+, 4·94; Ca^{2+}, 2·54; Mg^{2+}, 1·2; Cl^-, 127·83; $H_2PO_4^-$, 1·19; HCO_3^-, 25; (+)glucose, 11. The solution was kept at 37 °C and bubbled with 95 % O_2 and 5 % CO_2 (Cowie, Kosterlitz & Waterfield, 1978), giving a final pH of 7·50. Peristaltic pumps were used for delivering and removing the fluid from the perfusion chamber. Pre-incubation with diisopropylfluorophosphate (DFP) (500 μg ml^{-1}) was carried out when the acetylcholine released either spontaneously or by electrical stimulation was

assayed, or when exogenous acetylcholine was added to the perfusion fluid to measure histamine release.

Field stimulation was carried out by means of ring platinum electrodes; the parameters used were 10 Hz, 0·5 ms duration, which have been shown to produce a selective stimulation of parasympathetic nerve endings (Hutchinson, Kosterlitz & Gilbert, 1976). The preparations were stimulated twice in 1 min, each stimulation period being of 20 s duration; perfusates of 10 ml volume were collected at 2 min intervals for the acetylcholine and histamine assays. A sensitive pressure transducer connected to an ink-writing pen oscillograph was used to measure intraluminal pressure changes produced by the contraction of the circular muscle.

Assay of acetylcholine

Samples of perfusates originating from isolated vagally innerved guinea-pig auricles or from isolated rat intestinal muscle, both in resting conditions and after stimulation, were bioassayed for acetylcholine on the guinea-pig ileum, in the presence of morphine (10^{-6} M), eserine (3×10^{-6} M) and cyproheptadine (10^{-6} M). A 4 by 4 latin square arrangement was used for administration of the assay preparation. Acetylcholine authenticity was identified by (a) the specific antagonism by hyoscine, and (b) destruction by increase of pH to over 11.

Assay of histamine

In samples collected in a similar fashion, histamine was measured fluorimetrically using the method of Shore, Burkhalter & Cohn (1959) as modified by Lorenz, Reimann, Barth, Kusche, Meyer, Doenicke & Hutzel (1972). In some experiments the histamine assay was carried out in the same samples both through the fluorimetric assay and through the bioassay on the guinea-pig ileum in the presence of atropine and methysergide (Giotti, Guidotti, Mannaioni & Zilletti, 1966). The two methods gave comparable results. The authenticity of histamine was demonstrated (a) by recording the excitation and fluorescence spectra, (b) by thin layer chromatography (propanol/ammonia, and iodine), (c) by bioassay on guinea-pig ileum according to a two by two design (Schild, 1942).

Computer-aided analysis of mast cell metachromasia

Specimens of the right and left guinea-pig auricles and of rat ileum were fixed according to the procedure described by Mota (1959) and Enerbäck (1966) and stained with Toluidine Blue.

The analysis of mast cell granules metachromasia was carried out by computerized microdensitometry, a cytophotometrical technique which has been previously employed for evaluating the amount of stainable components of cells in histological samples (Van der Ploeg, Van den Broek, Smeulders, Vossepoel & Van Duijin, 1977; Brugal, Garbay, Giroud & Adelh, 1979; Harms, Gunzer, Aus, Rüter, Haucke & ter Meulen, 1979; Ploem, Verwoerd, Bonnet & Koper, 1979; Sahota, Ibaraki, Heywood, Farris & Van der Wereld, 1981).

Since the metachromatic constituents of mast cell cytoplasm are localized exclusively in the granules, the intensity of metachromasia indicates the amount and density of mast cell granules and hence their functional state.

The measurements were made on seventy-five mast cells randomly selected in five (right atrium) and six (intestine) histological sections 6 μm thick. The total area analysed in the various specimens is reported in Fig. 7.

The measurements were performed using an Apple II personal computer interfaced by a Teleraster card (Telecom, Turin, Italy) to a CCTV television camera (Hitachi HV-62E) applied to a light microscope with a 90 × oil-immersion objective. The images were visualized on a monitor at a 5000 × final magnification.

The Teleraster card allows a digitized image into sixty-four grey levels to be achieved.

The software was written by one of us (S. Bianchi) in Basic Applesoft language. It allowed us to standardize the transmission light, considering equivalent to 100% the light emerging from a tissue-free area of the histological slide and assuming it to correspond to the value of the white level. The different grey levels were obviously expressed in lower percentage values. The program moreover permitted us to scan the image plane. This operation was performed over square cytoplasmic areas, delimited by four lighting points at the vertices, which were superimposed on the monitor cell images. The displacement of the scanning areas was made by a joystick and their surface areas were adjusted by two Apple paddle buttons. The program allows a statistical and graphic elaboration of data.

Drugs

The chemicals used to prepare the solutions for the fluorimetric assay were of Suprapur quality, E. Merck, A.G. Acetylcholine chloride (batch number: 31F-0189) was obtained from Sigma, and *O*-phthaldialdehyde from B. D. H. Chemicals Ltd. Other drugs used were: histamine dihydrochloride, Calbiochem; atropine sulphate, Merck; eserine sulphate, Sigma; hyoscine hydrobromide, B. D. H.; tetrodotoxin (TTX), Sankyo; diisopropylfluorophosphate (DFP), Fluka; morphine sulphate, Merck; cyproheptadine hydrochloride, Merck.

RESULTS

Effects of cholinergic drugs on the response of guinea-pig auricles and rat ileum to parasympathetic stimulation

Maximal vagal stimulation of guinea-pig auricles resulted in an almost complete standstill, although in some preparations isolated contractile bursts could be observed. Consistently, at the end of the stimulation a rebound phenomenon appears, characterized by a net increase in rate and amplitude of contraction. Treatment with atropine (10^{-7}–10^{-5} M) inhibits, in a dose-dependent fashion, the negative inotropic and chronotropic responses to vagal stimulation, which were significantly enhanced in the presence of eserine (10^{-5} M).

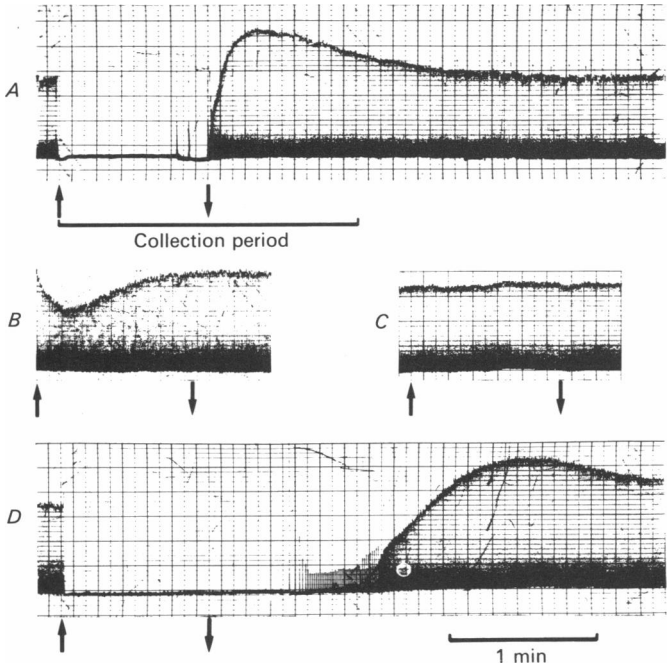

Fig. 1. Inotropic and chronotropic responses of guinea-pig auricles to 1 min vagal stimulation (20 Hz, 40 V, 1 ms); in *A*, control preparation; *B*, *C*, in the presence of atropine 10^{-7} and 10^{-5} M; *D*, in the presence of eserine 10^{-5} M. Arrows indicate the stimulation period and the horizontal bar the collection period.

Electrical field stimulation of rat ileum produced a sustained contractile response, which is dose-dependently reduced by tetrodotoxin and by atropine. Eserine potentiates the spasmogenic effect due to electrical stimulation.

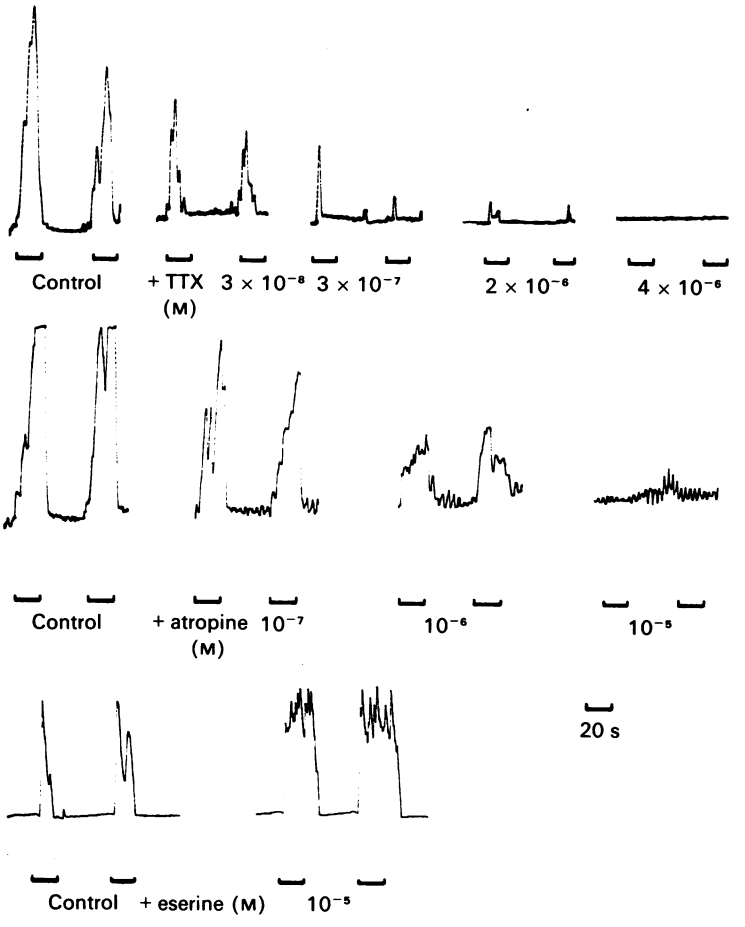

Fig. 2. Intraluminal pressure changes produced by electrical field stimulation of isolated rat ileum (10 Hz; 0·5 ms). The preparations were stimulated twice in 1 min for 20 s and the perfusates were collected every 2 min at a perfusion rate of 5 ml min^{-1}. Horizontal bar indicates the stimulation period.

Effect of parasympathetic stimulation on acetylcholine and histamine output by guinea-pig auricles and rat ileum

In guinea-pig auricles, the resting output of acetylcholine was measured in a 2 min sample at perfusion rates of 5 ml min^{-1} in the presence of eserine 10^{-5} M. A spontaneous overflow of acetylcholine was consistently found in the effluents of guinea-pig auricles; vagal stimulation caused a net increase of the acetylcholine output, the amounts of acetylcholine appearing in the perfusion fluid reaching the pre-stimulation levels within the two recovery periods measured.

Using the same design for the collection of the samples, a spontaneous release of acetylcholine was measured in isolated rat ileum pre-incubated with DFP (500 μg ml^{-1}). The output of acetylcholine evoked by electrical stimulation was significantly increased, with a prompt recovery to pre-stimulation values when the stimulus was turned off (Table 1).

Similar experiments were carried out to measure the output of histamine by

guinea-pig auricles and rat ileum, both in resting conditions and after stimulation, in the absence of acetylcholinesterase inhibitors. The results shown in Table 2 indicate that, both in guinea-pig auricles and rat ileum, a substantial amount of histamine appears in the perfusates under resting conditions, and that parasympathetic stimulation neatly increases the output of histamine, with a time course similar to that occurring with acetylcholine.

TABLE 1. Acetylcholine output from isolated guinea-pig auricles and isolated rat ileum in response to parasympathetic stimulation

Collection time (min)	Condition	Acetylcholine output ($pg\ min^{-1}\ mg^{-1}$ wet wt.) Guinea-pig auricles*	Rat ileum†
0–2	Spontaneous	480 ± 3·7	142 ± 1·4
2–4	Stimulated	670 ± 3·9	256 ± 3·6
4–6	Recovery	500 ± 2·9	216 ± 5·8
6–8	Recovery	490 ± 2·9	140 ± 4·3

* In the presence of eserine 10^{-5} M; the values are the means ± S.E. of mean of three experiments.
† Pre-incubated with DFP 500 $\mu g\ ml^{-1}$; the values are the means ± S.E. of mean of seven experiments.

TABLE 2. Histamine output from isolated guinea-pig auricles and isolated rat ileum in response to parasympathetic stimulation

Collection time (min)	Condition	Histamine output ($pg\ min^{-1}\ mg^{-1}$ wet wt.) Guinea-pig auricles	Rat ileum
0–2	Spontaneous	520 ± 47	121 ± 19
2–4	Stimulated	1492 ± 127	223 ± 33
4–6	Recovery	509 ± 65	133 ± 31
6–8	Recovery	495 ± 52	101 ± 35

The values are the means ± S.E. of mean of sixteen experiments.

Tissue histamine content was measured under resting conditions and after parasympathetic stimulation, both in guinea-pig auricles and rat ileum. None of these treatments caused a significant decrease in tissue histamine content, the histamine levels ranging from 8·1 ± 1·9 $\mu g\ g^{-1}$ fresh tissue in unstimulated auricles to 7·9 ± 2·4 $\mu g\ g^{-1}$ in stimulated preparations (mean values of twenty-five experiments); and from 9·5 ± 0·5 $\mu g\ g^{-1}$ in resting rat ileal segments to 10 ± 1·2 $\mu g\ g^{-1}$ in stimulated preparations (mean values of thirty experiments).

Experiments were carried out to establish whether the stimulation-evoked histamine output undergoes tachyphylaxis, and whether experimental conditions mimicking the physiological responses to nerve stimulation could reproduce the release of histamine. Table 3 shows that the resting and stimulation output of histamine is of the same order within three subsequent periods of stimulation, both in guinea-pig auricles and rat ileum.

Fig. 3 represents an experiment in which a sino-atrial lesion reproduces the

TABLE 3. Histamine output from isolated guinea-pig auricles and isolated rat ileum in response to repeated parasympathetic stimulation

Collection time (min)	Condition	Histamine output (pg min^{-1} mg^{-1} wet wt.) Guinea-pig auricles	Rat ileum
0–2	Spontaneous	420±34	121±16
2–4	Stimulated	808±32	215±20
4–6	Recovery	435±11	134±14
6–8	Recovery	415±22	131±17
18–20	Spontaneous	361±28	130±17
20–22	Stimulated	849±77	215±12
22–24	Recovery	382±45	136±12
24–26	Recovery	357±32	133±20
36–38	Spontaneous	342±34	128±13
40–42	Stimulated	846±19	223±14
42–44	Recovery	382±22	144±17
44–46	Recovery	370±24	133±23

The values are the means±S.E. of mean of six experiments.

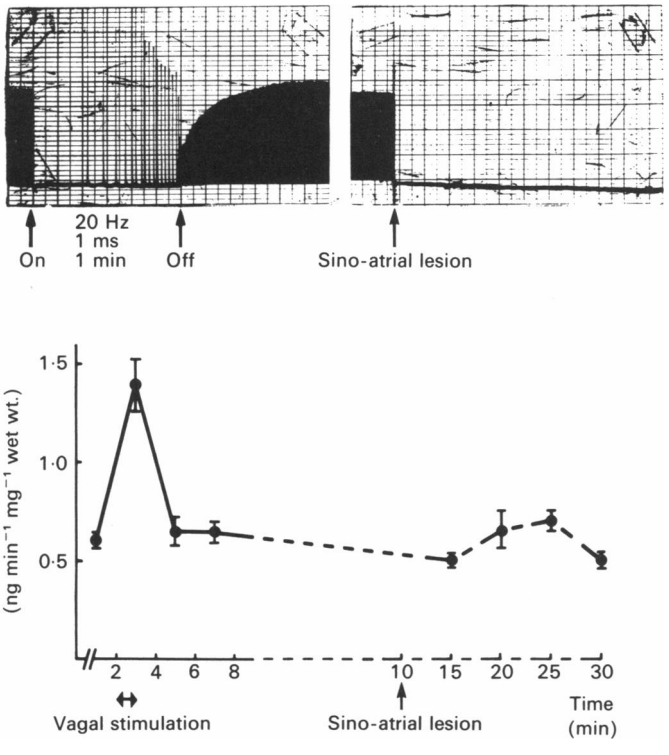

Fig. 3. Comparison between histamine output evoked by vagal stimulation and by sino-atrial lesion. The values are the means±S.E. of mean of four experiments.

mechanical events which follow the vagal stimulation (i.e. the standstill of both contraction and rate); however, under these circumstances, no increase of the release of histamine could be detected.

Effects of cholinergic drugs on the histamine output in guinea-pig auricles and rat ileum

When exogenous acetylcholine was added to the perfusion fluid of rat isolated ileal segments, after pre-incubation with DFP, the histamine output in the perfusates was increased over the resting output in a concentration-dependent fashion (Fig. 4). Similar results have been obtained in isolated guinea-pig hearts, in which acetylcholine, added to perfusates recirculated at a constant rate, almost doubled the amount of histamine appearing in the perfusate (Fantozzi *et al.* 1978*b*).

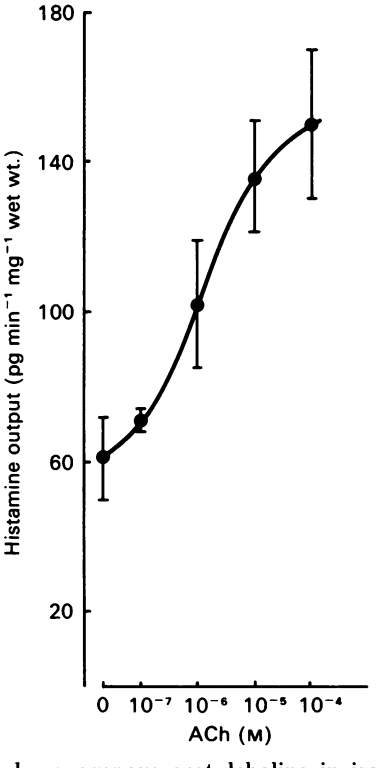

Fig. 4. Histamine release by exogenous acetylcholine in isolated rat ileum after pre-incubation for 150 min with DFP (500 μg ml^{-1}). The values are the means $\pm$ S.E. of mean of seven experiments.

In a series of experiments, we tested whether the resting and stimulated histamine output was influenced by atropine. When a variety of concentrations of atropine were present in the incubation medium, the resting histamine release of each collection period was not significantly different than in the control experiments, both in guinea-pig auricles and in rat ileum. However, atropine produced a dose-related decrease in the release of histamine from the field-stimulated rat ileum, and from guinea-pig auricles, when the vagus nerve was stimulated (Fig. 5).

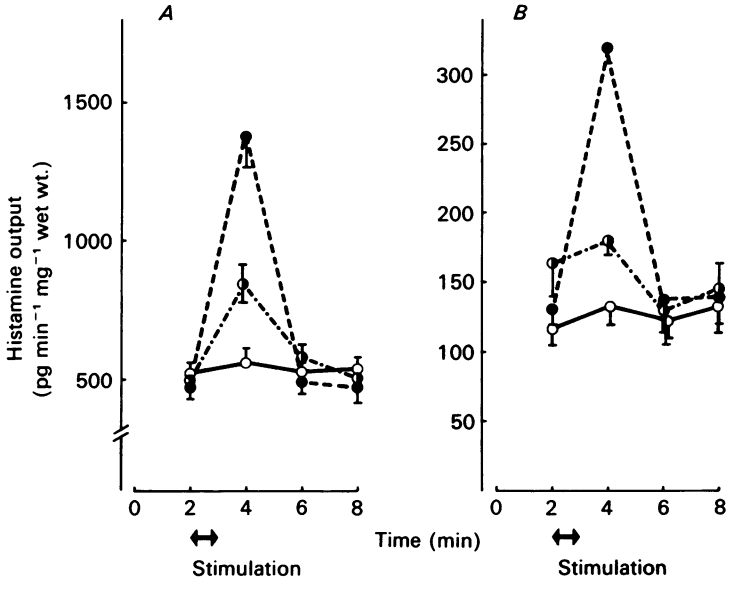

Fig. 5. Effect of atropine on histamine output by parasympathetic stimulation in isolated guinea-pig auricles (*A*) and isolated rat ileum (*B*). ●, control; ◑, atropine 10^{-6} M; ○, atropine 10^{-5} M. Atropine was added to the perfusion fluid 20 min before nervous stimulation. The results are the means ± S.E. of mean of seven experiments.

The ability of eserine to influence the resting output of histamine, and the histamine release evoked by parasympathetic stimulation was also determined. As shown in Fig. 6, the presence of eserine did not alter the spontaneous histamine release, but significantly extended the time course of histamine release in response to parasympathetic stimulation, both in guinea-pig auricles and in rat ileum.

Differential evaluation of mast cell metachromasia in guinea-pig auricles and rat ileum

Differential evaluations were made of mast cell granules metachromasia in the guinea-pig auricles and in rat ileum, in preparations maintained *in vitro* and not submitted to stimulation, and in preparations kept *in vitro* for a corresponding time, but submitted to parasympathetic stimulation. The over-all intensity of mast cell granules metachromasia was measured by the distribution of the transmission light, using the computer-aided microdensitometry referred to under Methods. In mast cells from unstimulated guinea-pig right auricles, the average transmittance was 54 ± 14·7 %, and 81·8 ± 11·7 % in mast cells from stimulated preparations (the value of the light emerging from a tissue-free area of the histological slide being equivalent to 100 %); the corresponding figures of the average transmittance in rat intestinal mast cells were 51·8 ± 14·1 % (resting) and 65·2 ± 12·8 % stimulated (Fig. 7).

The analysis reported in Fig. 7 allows the quantitative evaluation of the real and cumulative transmission levels distribution, showing that, in both preparations, the percentage distribution areas and the cumulative distribution curves were statistically different, when comparing the resting to the stimulated preparations. This may

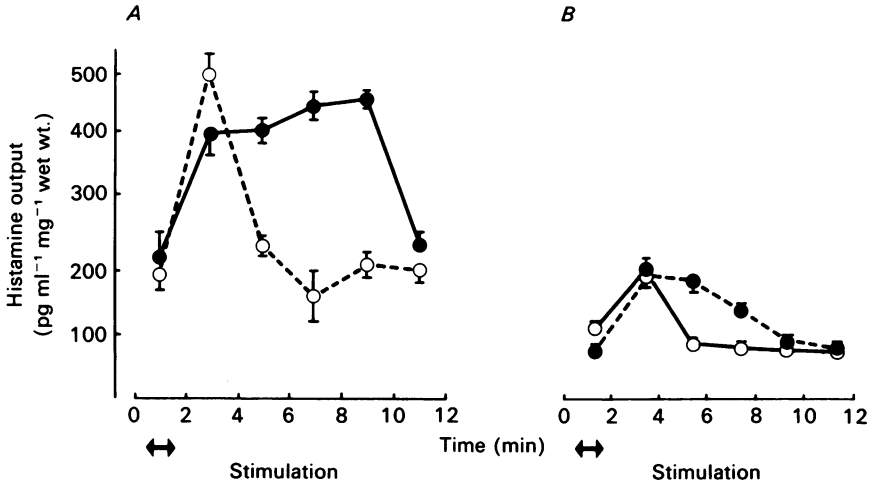

Fig. 6. Effect of eserine (10^{-5} M) on histamine output by parasympathetic stimulation in isolated guinea-pig auricles (*A*) and isolated rat ileum (*B*). ○, control; ●, eserine 10^{-5} M; eserine was added to the perfusion fluid 20 min before nervous stimulation. The results are the means ± S.E. of mean of seven experiments.

account for the graded level of mast cell granules metachromasia occurring under physiological conditions, and for the significant diminution of the intensity of mast cell granules metachromasia after vagal stimulation.

DISCUSSION

The experiments reported here have demonstrated that maximal stimulation of parasympathetic nerve endings, either preganglionic, through the vagus nerve, or post-ganglionic, through the selective field stimulation of the nerve terminals, is associated with a significant increase of the physiological overflow of acetylcholine in the perfusion fluid.

In both the preparations we have selected as experimental models, the release of acetylcholine evoked by parasympathetic stimulation was accompanied by a quantitatively comparable histamine release. A cholinergic link between the parasympathetic stimulation and the observed release of histamine is suggested by the following evidence: (a) atropine competitively inhibits both the physiological responses and the release of histamine after parasympathetic stimulation; (b) eserine potentiates the physiological effects due to electrical stimulation, and also extends the time course of the histamine output in response to electrical stimulation; (c) atrial standstill obtained through a non-parasympathetic stimulus (i.e. sino-atrial lesioning) was not accompanied by a significant release of histamine.

As far as the source of the released histamine is concerned, it is possible that the amounts of histamine appearing in the perfusates collected from guinea-pig auricles and rat ileum may stem from (a) the nerve endings; (b) from tissue mast cells; (c) from non-mast-cell histamine stores.

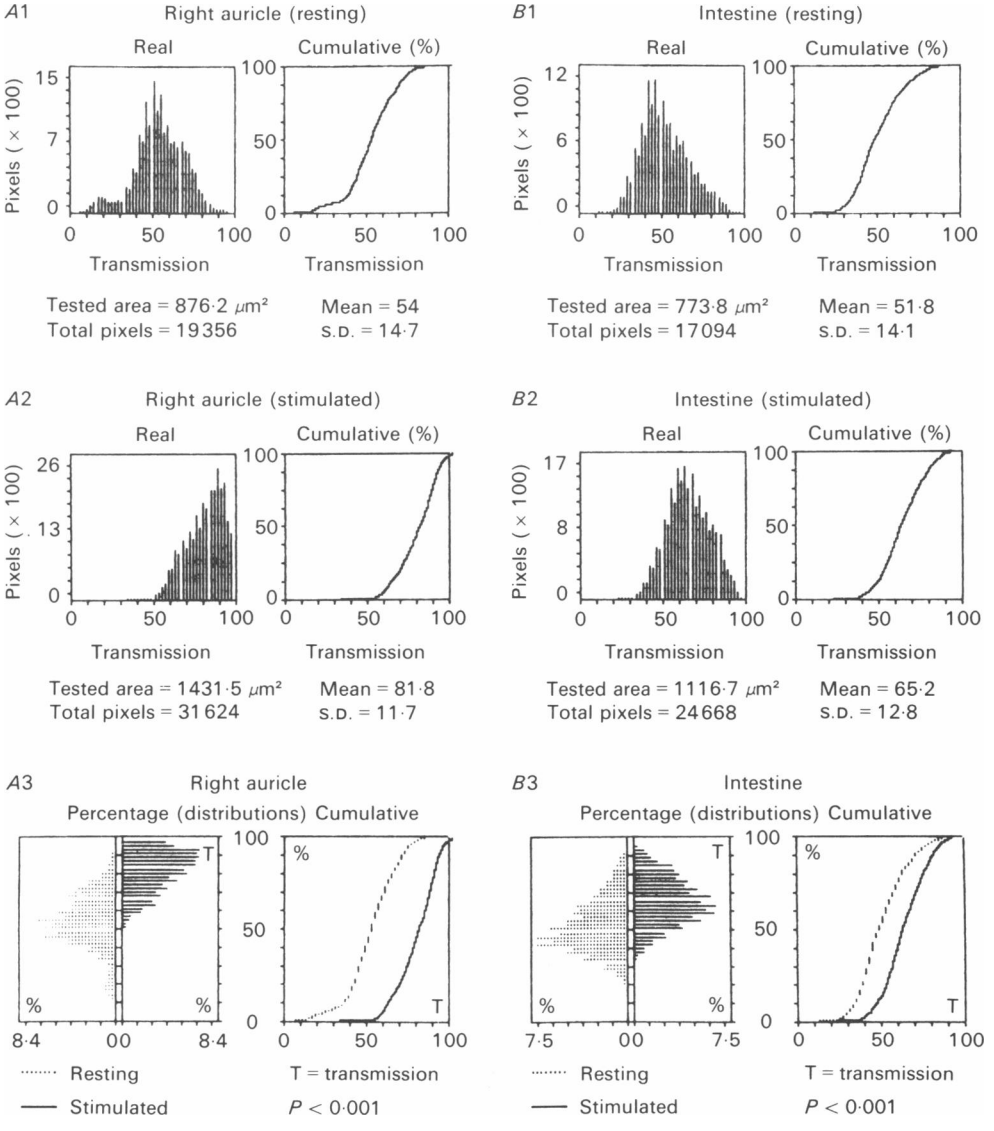

Fig. 7. Intensity of mast cells granules metachromasia measured by the transmission light. *A*1, transmission levels distribution of mast cell granules from resting right auricle of the guinea-pig; *A*2, transmission levels distribution of mast cell granules from stimulated right auricle of the guinea-pig; *A*3, comparison between the two distributions. *B*1, transmission levels distribution of mast cell granules from resting rat intestine; *B*2, transmission levels distribution of mast cell granules from stimulated rat intestine; *B*3, comparison between the two distributions.

In cardiac preparations, it is unlikely that the resting and stimulated histamine output may be accounted for by a release of histamine stored in cardiac cholinergic fibres of the 'autonomic ground-plexus' (Hillarp, 1959). Although high concentrations of histamine have been associated with parasympathetic nerves (Von Euler, 1966),

our present results using atropine and eserine favour a neurogenic histamine release. In fact the inhibition by atropine of the histamine release, and its potentiation by eserine suggest a post-synaptic muscarinic modulation of histamine output.

In intestinal preparations it is possible that a source of the histamine output could be represented by a pool of histamine stored in the parasympathetic nerve endings. Recent experiments have looked for a neuronal store of histamine in rat intestine, using an immunocytofluorimetric method (Håkanson, Ekblad, Wahlestedt & Sundler, 1984). However, the inhibitory effect of atropine and the potentiation of histamine release by eserine are again in favour of a post-synaptic muscarinic modulation of histamine output.

We cannot rule out, at the present time, that acetylcholine, which is released after electrical stimulation, would in turn evoke the release of histamine from a non-mast-cell pool. However, the very existence of a non-mast-cell histamine pool in cardiac and intestinal tissues is open to some doubts. It is now generally accepted that essentially all the histamine in rat intestine is stored in mucosal mast cells, which have been recognized as a cell line separate from connective tissue mast cells, from which they differ in biochemical, morphological and functional respects (Newson, Dahlström, Enerbäck & Ahlman, 1983). In the guinea-pig heart, a close relationship has been found between the histamine levels and the mast cell numbers in the different chambers (Guidotti, Zilletti & Giotti, 1967); in a mutant strain of mice genetically deprived of mast cells only negligible traces of histamine have been found in the heart (Yamatodani, Maeyama, Watanabe, Vada & Kitamura, 1982).

Our present experiments indicate a net diminution of mast cell granules metachromasia after vagal stimulation both in the isolated guinea-pig auricles, and in the isolated rat ileum. We are, consequently, tempted to assume that parasympathetic stimulation releases acetylcholine from the specific nerve terminals, which in turn triggers histamine secretion, presumably from tissue mast cells.

The hypothesis of a cholinergic histamine release from mast cells is in keeping with the trend which considers mast cells not only as capable of reacting to noxious stimuli, but also as a sensitive component of more physiological events, such as vasodilatation, cardiac contractility, intestinal secretion and peristalsis. In a similar model, antidromic impulses in substance-P-containing primary afferent neurones would release substance P which, in turn, could release histamine from mast cells and produce vasodilatation (Foreman & Jordan, 1983). In our model, the cholinergic histamine release could contribute to maintain local homoeostasis in the heart and in the ileum (i.e. nutrition, secretion, contractility), beside influencing pathological events such as cholinergic asthma and cholinergic urticaria (Harries, O'Brien, Burge & Pepys, 1979).

In this context, it is conceivable that the graded levels of mast cell granules metachromasia observed under resting conditions, and the decrease in mast cell granules metachromasia occurring after parasympathetic stimulation could represent a tonic and phasic secretion of granular materials, suitable to afford a local hormonal action.

A functional relationship between nerve and tissue mast cells has been suggested by the contiguity between mast cells and nerve terminals (Stach, Weiss & Radke, 1975) and the degranulation of mast cells caused by antidromic stimulation of cutaneous nerve (Kiernan, 1972). It has also been suggested that peripheral autonomic

neurones can exert their action on effector cells by transmitter release even without true synapses, provided that the distance of the autonomic gaps does not exceed 100 nm (Burnstock & Costa, 1975). If this holds true also for mast cells, they may possibly be influenced by the axons running in bundles very close to the cells. Recent evidence is in favour of a direct innervation of mast cells. In fact, microscopic patterns suggestive of true synapses between nerve fibres and mast cells have been described by Wiesner-Menzel, Schulz, Vakilzadeh & Czarnetzki (1981) in a subungual solitary glomus tumor in man, and by Heine & Forster (1975) in human subcutis and dog myocardium. Moreover, in a recent study (Newson *et al.* 1983) mucosal mast cells from terminal ileum of rats demonstrated the presence of numerous nerve terminals or boutons seemingly in direct contact with their plasma membrane. Under the electron microscope, membrane specialization highly suggestive of true synapses was observed and the nature of the boutons identified as adrenergic and/or cholinergic by the shape and electron density or their vesicles (Newson *et al.* 1983).

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